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'Giardia' Detection in Water Systems

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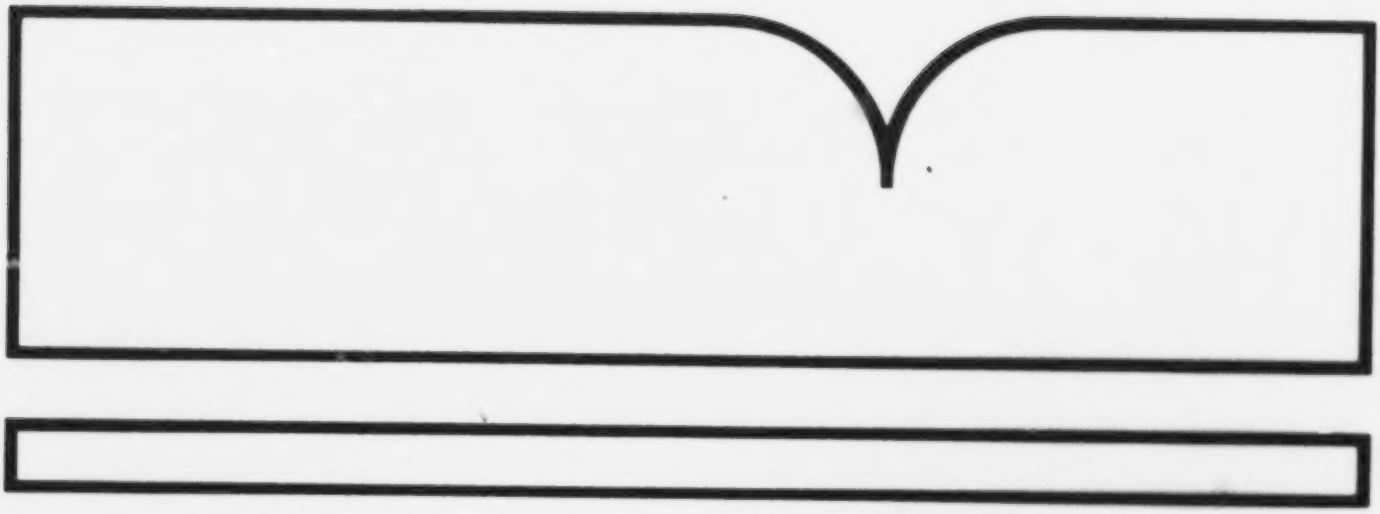

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GIARDIA DETECTION IN WATER SYSTEMS

by

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A method has been developed combining immunofluorescence and phase-contrast to microscopically locate and identify Giardia cysts among particulates filtered from water samples. Selective staining of cysts with anti-Giardia antiserum and a fluorochrome enhances the contrast between cysts and other sample constituents and allows quick location of cysts by their green fluorescence. Their identity can then be confirmed with phase-contrast observation of size, shape, and internal morphology. This method was successfully tested during recent outbreaks in Pennsylvania and Massachusetts. However, when chlorinated water samples were tested, confirmation of presumptive Giardia cysts was difficult or impossible. Subsequent laboratory exposure of Giardia cysts to various chlorine levels showed that although these cysts could still be detected by fluorescence, their internal morphology had been destroyed. These methods for detecting and identifying Giardia cysts may be useful in confirming outbreaks, providing guidance on treatment processes, and establishing baseline data on the prevalence of Giardia cysts in water supplies.

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ABSTRACT

A method has been developed combining immunofluorescence and phase-contrast to microscopically locate and identify Giardia cysts among particulates filtered from water samples. Selective staining of cysts with anti-Giardia antiserum and a fluorochrome enhances the contrast between cysts and other sample constituents and allows quick location of cysts by their green fluorescence. Their identity can then be confirmed with phase-contrast observation of size, shape, and internal morphology. This method was successfully tested during recent outbreaks in Pennsylvania and Massachusetts. However, when chlorinated water samples were tested, confirmation of presumptive Giardia cysts was difficult or impossible. Subsequent laboratory exposure of Giardia cysts to various chlorine levels showed that although these cysts could still be detected by fluorescence, their internal morphology had been destroyed. The immunofluorescence method for detecting and identifying Giardia cysts may be useful in confirming outbreaks, providing guidance on treatment processes, and establishing baseline data on the prevalence of Giardia cysts in water supplies.

INTRODUCTION

Giardia has been implicated in more than 80 waterborne outbreaks in the U.S. since 1965 (1). Detection of the cyst form of this organism in water systems is difficult since, unlike bacteria, cyst numbers cannot be amplified by in vitro cultivation. Reliable indicators for this organism do not yet exist. Therefore, cysts must be detected by direct microscopic evaluation of water sample concentrates. Frequently only small numbers of cysts are found among large quantities of other microbes and debris. An improved method has been developed (2) to detect *Giardia* cysts. This method uses fluorescently-labeled antibody to selectively stain the cysts. This causes the cysts to glow green when illuminated by UV light. The technique allows cysts to be quickly located amongst the unstained microbes and debris. The presumptive identification based on fluorescence, size, and shape can then be confirmed using phase-contrast observation. This paper will first briefly describe the process of recovering *Giardia* cysts from water samples. Next it will summarize the immunofluorescence/phase-contrast method for detection and identification of cysts. And, finally, it will show how the method was field tested.

Recovery of Giardia Cysts from Water Samples

Recovery and detection of *Giardia* cysts is accomplished by a tentative or reference method developed by consensus in 1980 (3, 4). Briefly, at least 100 gallons of raw or finished water is passed through a 1 µm nominal porosity yarn-wound filter. The retained particulates are then extracted by washing the filter's fibers. The particulates are concentrated by sedimentation. Finally, the *Giardia* cysts and other particulates of similar bouyant density are recovered using isopycnic centrifugation. In this technique, particulates of similar density are separated from lower and higher density particulates by floating the particulates within a step gradient composed of fluids of increasing specific gravity. The recovered particulates are then usually stained with iodine prior to microscopic examination. Positive identification of *Giardia* cysts is first made by size (10-20 µm long x 5-15 µm wide) and shape (oval or oval-round). Observation of at least two characteristic internal features such as nuclei, median bodies, or axonemes confirms the identity (3, 4, 5).

Immunofluorescence-Phase Method for Detection and Identification

Iodine staining of water sample sediments usually results in a low contrast between *Giardia* cysts and the rest of the sample particulates. This makes detection of small numbers of cysts in heavily contaminated samples very difficult. Selective staining of *Giardia* cysts with anti-*Giardia* antiserum and a fluorochrome enhances the contrast between cysts and the other particulates and thus allows easier and faster location of cysts (2, 6). Also, because the method is performed on membrane filters, more of the sample can be examined per slide than is examined using the reference method.

Figure 1 shows an overview of the immunofluorescence method for detecting *Giardia* cysts in water samples (see Reference 2 for details). In this procedure, portions of the water sample concentrate are deposited

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on membrane filters held in a vacuum manifold. Reagents and rinse fluids are added sequentially and removed through the filter by suction. Counterstaining, dehydration by alcohol, and mounting in glycerol enable visualization of internal features by phase-contrast microscopy. Positive controls consisting of known G. lamblia cysts are applied to blank filters to check the method and to acclimate the observer's eye to the proper fluorescence, size, and shape that must be detected.

In all water samples tested so far, organisms other than Giardia have been found to autofluoresce yellow or red and are easily distinguished from the apple green fluorescein stain. Cross reactivity of the antiserum with other microorganisms has been observed, but the majority of these organisms which fluoresce green are easily disregarded, as they do not fit the size and shape requirements. In all water samples tested by this method only two cross reacting organisms of the same shape and size as Giardia cysts have been observed. These forms (round or egg-shaped) were easily distinguished from Giardia cysts using phase-contrast examination of internal morphology. Objects having the correct size, shape, and characteristic fluorescence of Giardia cysts, but which lack recognizable internal morphology, are reported as Giardia cyst-like objects.

Field Testing of the Immunofluorescence Method

From December, 1983 to December, 1985, 50 surface water samples from giardiasis outbreaks in Pennsylvania and Massachusetts were tested with this method. The majority of samples (76%) had a collection volume of 200-500 gallons of water. Results of the sample analyses are summarized in Table 1. Of the 27 raw water samples examined, 63% were positive for Giardia. And, of 23 finished water samples, 27% were positive. All of the raw water samples were from surface waters, mainly reservoirs and rivers. Finished water samples were taken anywhere from the treatment plant to private taps.

Not all of each sample concentrate was examined in every case. But, in those samples where no cysts or cyst-like objects were found, the portion of sample concentrate examined always represented at least 100 gallons of original water sample. Samples in which no Giardia cysts were detected were not declared as absolutely negative since the recovery efficiency of the processing and detection methods are yet unknown. The number of presumptive Giardia cysts detected varied widely depending on the water source. In the portions of positive raw samples examined, an average of 131 cysts (range 2-1359) were detected, whereas finished waters yielded an average of 13 cysts (range 7-61).

The inability to confirm highly suspicious Giardia cyst-like objects presented a special problem. Frequently their internal appearance was granular, crenated, or composed of vacuoles. Several samples in which these objects were found had been treated with high levels of chlorine. Subsequent laboratory exposure of G. lamblia and G. muris cysts to chlorine for up to 48 hours revealed that their internal morphology, but not their fluorescence was adversely affected by chlorine (7). After 48 hours, even at the lowest Cl_2 levels, although the cysts still fluoresced, the majority had no identifying internal characteristics. Vacuoles and grains were frequently observed within cysts treated with chlorine for

shorter time intervals. Thus, ambiguous test results may occur when chlorinated water samples are tested.

SUMMARY

We have come a long way since the Rome, New York outbreak (8) when one million gallons of water was sampled to find one Giardia cyst. Improved Giardia analytical methods, such as the reference and the immunofluorescence method, have increased awareness of the prevalence of Giardia in water supplies. But many questions remain unanswered. These tests cannot give us the absolute numbers of cysts in our water supplies, nor can they yet tell us the viability, infectivity, or the source of the cysts. However, after using these methods, positive results can confirm that an outbreak was waterborne. This information then can provide guidance on treatment processes and can be used to establish baseline data on the prevalence of Giardia cysts in water supplies.

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TABLE 1. ANALYSIS OF PENNSYLVANIA AND MASSACHUSETTS WATER SAMPLES FOR *GIARDIA* CYSTS

	Sample Source	
	Raw Water	Finished Water
Total number of samples examined	27	23
% containing positively identified *Giardia* cysts	63	27
% containing unconfirmed *Giardia* cyst-like objects	11	41
% with no *Giardia* cysts or cyst-like objects detected	26	32

FIGURE 1. SUMMARY OF IMMUNOFLUORESCENCE-PHASE CONTRAST METHOD FOR GIARDIA DETECTION IN WATER

Portion of Water Sample Concentrate Deposited onto Membrane Filters
Treat with Rabbit Anti-Giardia Antiserum
Treat with Goat Anti-Rabbit Antibody Fluorescein
Add Evans Blue Counterstain
Dehydrate using Alcohol Series (10%-Absolute)
Mount Membrane Filter Mounted in Glycerol
Scan with UV at Low Magnification for Detection of Green Fluorescing Objects of Correct Size and Shape
View with Phase Contrast at High Magnification for Identification of Internal Morphological Characteristics

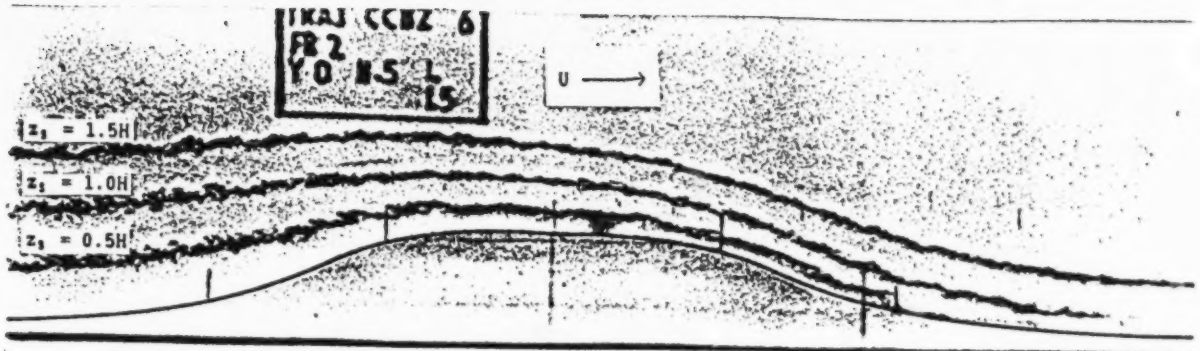

Figure 1. Side view of centerline trajectories; Fr = 2.0.

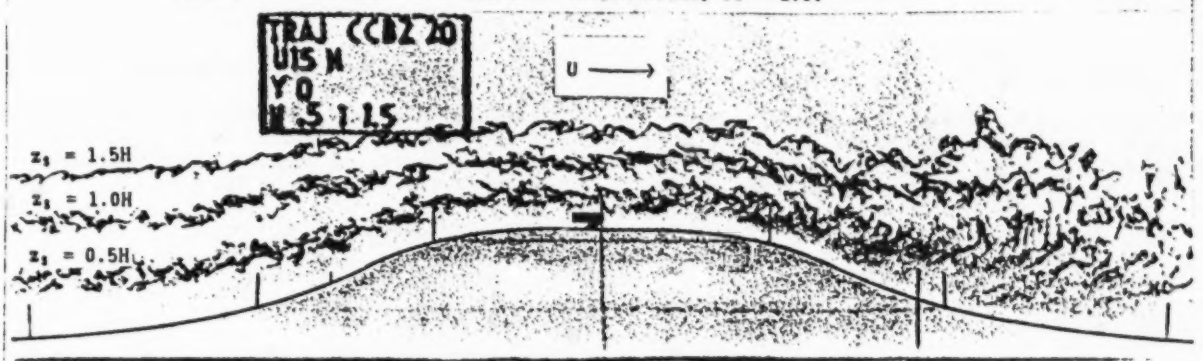

Figure 2. Side view of centerline trajectories; Fr = ∞.

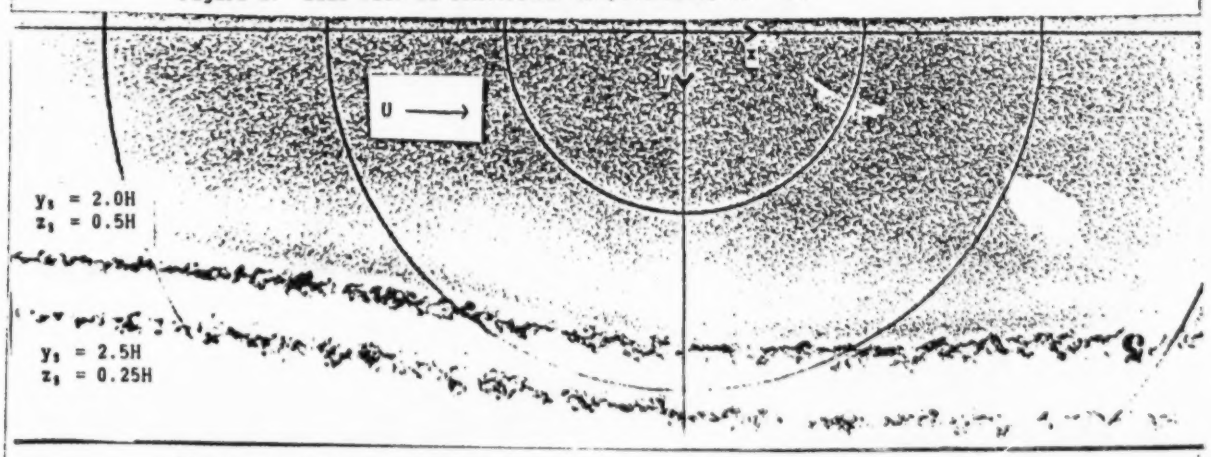

Figure 3. Bottom view of trajectories for the laterally offset source positions; Fr = 2.0.

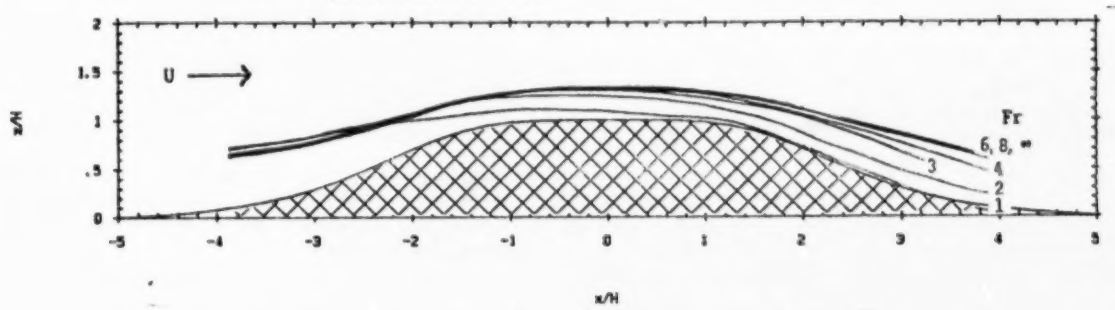

Figure 4. Averaged, computed, centerline trajectories; z_s = 0.5H.

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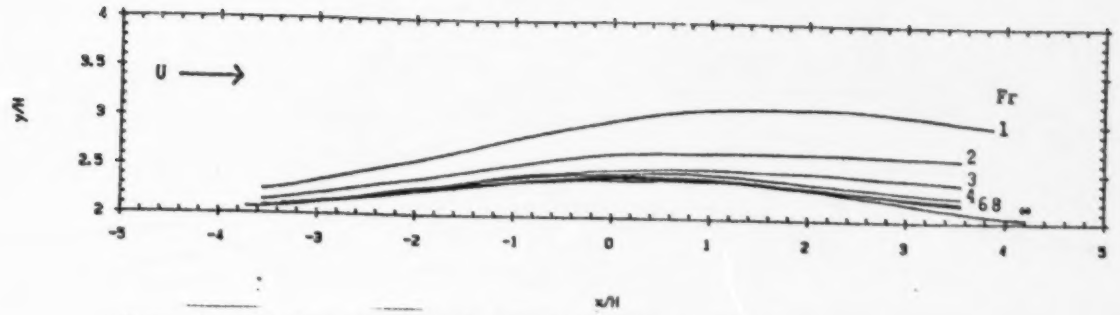

Figure 5. Top view of averaged, computed, trajectories for laterally offset sources; y_s = 2.0H, z_s = 0.5H.

Table 1
Parameters For Centerline Releases

z_s is the source height, Fr is Froude number, z(0,0) is the streamline height over the hill center, η_{min} is the closest approach to the hill surface, and $x|\eta_{min}$ is the position of η_{min}.

z_s/H	Fr	z(0,0)/H	η_{min}/H	$x\|\eta_{min}/H$
0.5	1	1.09	0.00	3.87 *
0.5	2	1.24	0.15	3.87 *
0.5	3	1.30	0.24	1.24
0.5	4	1.33	0.28	1.32
0.5	6	1.33	0.30	-1.36
0.5	8	1.34	0.30	-1.55
0.5	∞	1.32	0.30	-1.21
1.0	1	1.30	0.16	3.87 *
1.0	2	1.53	0.37	1.47
1.0	3	1.62	0.58	1.23
1.0	4	1.66	0.61	0.93
1.0	6	1.66	0.61	-1.20
1.0	8	1.67	0.62	-1.38
1.0	∞	1.66	0.64	-1.30
1.5	1	1.56	0.40	1.47
1.5	2	1.83	0.66	1.47
1.5	3	1.95	0.89	1.24
1.5	4	1.93	0.90	1.10
1.5	6	1.99	0.97	1.11
1.5	8	1.99	0.97	-0.85
1.5	∞	1.96	0.94	-1.10

* the nearest approach observed occurred at the farthest downstream observation

Table 2
Parameters For Offset Releases

Fr is Froude number, $\Delta y|x=0$ is lateral deflection at x=0, Δy_{max} is maximum lateral deflection, $x|\Delta y_{max}$ is its location, η_{min} is closest approach to the hill surface, and $x|\eta_{min}$ is its position.

Fr	$\Delta y\|x=0/H$	$\Delta y_{max}/H$	$x\|\Delta y_{max}/H$	η_{min}/H	$x\|\eta_{min}/H$
		(y_s = 2.5H, z_s = 0.25H)			
1 *	----	1.08	-1.22	0.35	-1.35
2	0.76	0.89	3.55	0.24	-0.14
3	0.61	0.63	0.48	0.25	-0.70
4	0.53	0.54	0.43	0.21	-0.85
6	0.50	0.50	0.07	0.20	-0.78
8	0.50	0.50	0.51	0.24	-0.65
∞	0.47	0.48	0.52	0.20	-0.75
		(y_s = 2.0H, z_s = 0.5H)			
1	0.99	1.14	2.09	0.30	3.87
2	0.66	0.69	1.63	0.44	-0.36
3	0.49	0.51	0.62	0.36	-0.42
4	0.46	0.47	0.64	0.34	0.14
6	0.42	0.42	0.07	0.35	-0.50
8	0.43	0.44	0.27	0.39	-0.63
∞	0.39	0.40	0.81	0.35	0.32

* the plume left the field of view at x = 1.22H.

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